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Mutation Analysis of PTEN/MMAC1 in Acute Myeloid Leukemia

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Recently, a putative tumor suppressor gene, PTEN/MMAC1, has been identified at chromosome 10q23.3, which encodes a 403 amino acid dual-specificity phosphatase containing a region of homology to tensin and auxillin. Somatic mutations of the PTEN/MMAC1 gene have been identified in a number of cancer cell lines and primary cancers. Mutations in PTEN/MMAC1 are most frequently found in advanced cancers. To evaluate the role of the PTEN/MMAC1 gene in leukemia, bone marrow and/or peripheral blood from 62 acute myeloid leukemia (AML) patients, 5 hemopoietic cell lines (HL60, U937, Raji, KG-1, K562), and 30 normal controls were analyzed. The results showed aberrant PTEN/MMAC1 transcripts in 15 of the 62 (24%) AML patients, 4 of the 5 cell lines (80%), and 4 of the 30 (13%) normal controls. As in our previous study of TSG101, the abnormal transcripts may result from aberrant RNA splicing as evidenced by the presence of both these aberrant transcripts and normal full length transcripts in all specimens examined. Loss of heterozygosity (LOH) analysis and PCR-SSCP of the entire coding region showed that none of the AML cases had LOH or mutation. Only one frameshift mutation at codon 130 (insertion of CCCG) with premature termination of coding sequence was observed in the U937 cell line. Our results indicate that the PTEN/MMAC1 gene may play a role in a small percentage of AML, but its significance needs to be further evaluated. Am. J. Hematol. 63:170–175, 2000.

Key words: PTEN/MMAC1 gene; acute myeloid leukemia; RT-PCR; loss of heterozygosity

INTRODUCTION

The molecular pathogenesis of cancers including leukemias involves a stepwise accumulation of mutations affecting both cellular oncogenes and tumor suppressor genes [1–3]. The major chromosome aberrations in leukemia are specific translocations juxtaposing genes that activate oncogenes or create novel fusion genes [4–6]. Mutations or deletions in tumor-related genes are frequently accompanied by loss of the remaining allele or alleles (loss of heterozygosity, LOH), leading to the inactivation of these tumor suppressor genes, which is thought to be involved in a multi-step accumulation of gene alterations crucial to leukemogenesis [4,6–8].

Recently, a putative tumor suppressor gene, PTEN/MMAC1, has been identified at chromosome 10q23.3 [9–11]. Somatic mutations of the PTEN/MMAC1 gene have been identified in a number of cancer cell lines and primary cancers, and most frequently found in advanced cancers [9,10]. On the basis of such findings, PTEN/MMAC1 has been considered a candidate tumor suppressor gene that may play a role in varied malignancies.

Chromosome 10q23.3 is deleted in a variety of primary tumors including myeloid leukemia [5,9,10]. Homozygous deletion of the PTEN/MMAC1 gene has also been reported in some tumor cell lines [9,10,12]. Therefore, in order to elucidate the role of PTEN/MMAC1 in leukemogenesis, 62 AML cases, 5 hemopoietic cell lines,

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TABLE I. Oligonucleotide Primers for Studying the PTEN/MMAC1 Gene

RT-PCR and cDNA sequencing primers		
1st PCR	M1u	5′-AGAGCCATTTCCATCCTGCA-3′
	M1d	5′-GTGTCAAAACCCTGTGGATG-3′
Nested PCR	M2u	5′-CTCCTCCTTTTTCTTCAGCC-3′
	M2d	5′-TGACACAATGTCCTATTGCC-3′
	M3u	5′-CAAGAGGATGGATTCGAC-3′

	Primers for PCR-SSCP analysis	
	Forward primer	Reverse primer
Exon 1	5′-CTCCTCCTTTTTCTTCAGCC-3′	5′-ATATGACCTAGCAACCTGACCA-3′
Exon 2	5′-TGACCACCTTTTATTACTCCA-3′	5′-TAGTATCTTTTTCTGTGGCTTA-3′
Exon 3	5′-ATAGAAGGGGTATTTGTTGGA-3′	5′-TCCTCACTCTAACAAGCAGATA-3′
Exon 4	5′-TTCAGGCAATGTTTGTTA-3′	5′-TTCGATAATCTGGATGACTCA-3′
Exon 5	5′-GCAACATTTCTAAAGTTACCTA-3′	5′-TCTGTTTTCCAATAAATTCTC-3′
Exon 6	5′-GAGTAACTATTCCCAGTCAGA-3′	5′-TAATTTGTTCAAATGCTTCAGA-3′
Exon 7	5′-ATCGTTTTTGACAGTTTG-3′	5′-CCAATGAAAGTAAAGTACA-3′
Exon 8	5′-AGGTGACAGATTTTCTTTTTTA-3′	5′-TAGCTGTACTCCTAGAATTA-3′
Exon 9	5′-GTTCATCTGCAAAATGGA-3′	5′-TGGTAATCTGACACAATGTCCTA-3′

and 30 normal controls were studied. Mutation analysis was performed by sequencing the nine exons and adjacent intronic splice-junction regions of PTEN/MMAC1. LOH analysis in the 10q23 region and the RNA transcript of the PTEN/MMAC1 gene in specimens from AML and hemopoietic cell lines were also examined.

MATERIALS AND METHODS

Tumor Samples and Tumor Cell Lines

Sixty-two specimens of bone marrow and/or peripheral blood were obtained from patients diagnosed with AML at the Kaohsiung Medical College Hospital from 1992 through 1996. All the cases were AML de novo, from 18 to 76 years old. AML patients were classified according to French-American-British criteria as follows: M1, ten patients; M2, fourteen; M3, fifteen; M4, eleven; M5, eight; M6, four patients. Cytogenetic assessment was done in 54 of 62 cases, none had a chromosome 10 or 10q deletion. In addition, 5 hemopoietic cell lines (HL60, U937, Raji, KG-1, K562) and peripheral blood samples from 30 normal control were also studied. Hair follicles from 20 of the 62 AML patients were collected to evaluate constitutional DNA for LOH analysis.

Genomic DNA Preparation

Total genomic DNA was isolated and extracted from bone marrow or peripheral blood leukocyte of the subjects as described previously [13].

RNA Extraction and Reverse Transcription

Total RNA was purified as described previously [14] or using a commercial kit ($TRI_{zol}^{®}$ Reagent, GIBCO-BRL, NY). The extracted RNA was treated with RNase-free DNase I. cDNA was synthesized from 1 μg of total RNA. Reverse transcription was performed in a 20 μL final volume containing 1 μg RNA, 0.5 μg random primers (10 mers), 10 mM dithiothreitol, 0.5 mM dNTPs, 5 units RNasin (Promega, Madison, WI), 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM $MgCl_2$, and 200 units Moloney Murine Leukemia Virus reverse transcriptase. The reaction was first denatured for 5 min at 95°C and incubated at 37°C for 60 min. The reaction was then stopped by heat inactivation at 95°C for 5 min.

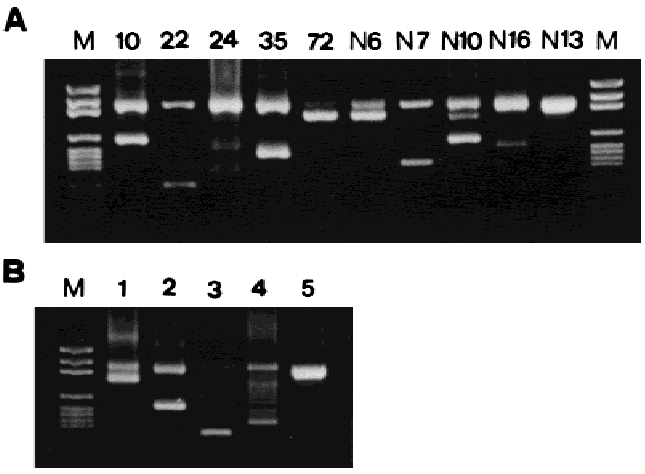

Fig. 1. RT-PCR analysis of the expression of the PTEN/MMAC1 gene. (A) Abnormal transcripts are noted in AML patients (cases 10, 22, 24, 35, and 72) and normal controls (N6, N7, N10, N16). (B) Abnormal transcripts in cell lines. Lane 1, U937; lane 2, Raji; lane 3, HL60; lane 4, KG-1; lane 5, K562. M represents pGem marker.

RT-PCR and cDNA Sequencing

Nested PCR was carried out using primers flanking the full coding sequence of the PTEN/MMAC1 cDNA, as shown in Table I. A 1-μL aliquot of cDNA was used for the first PCR amplification for 30 cycles, using primers,

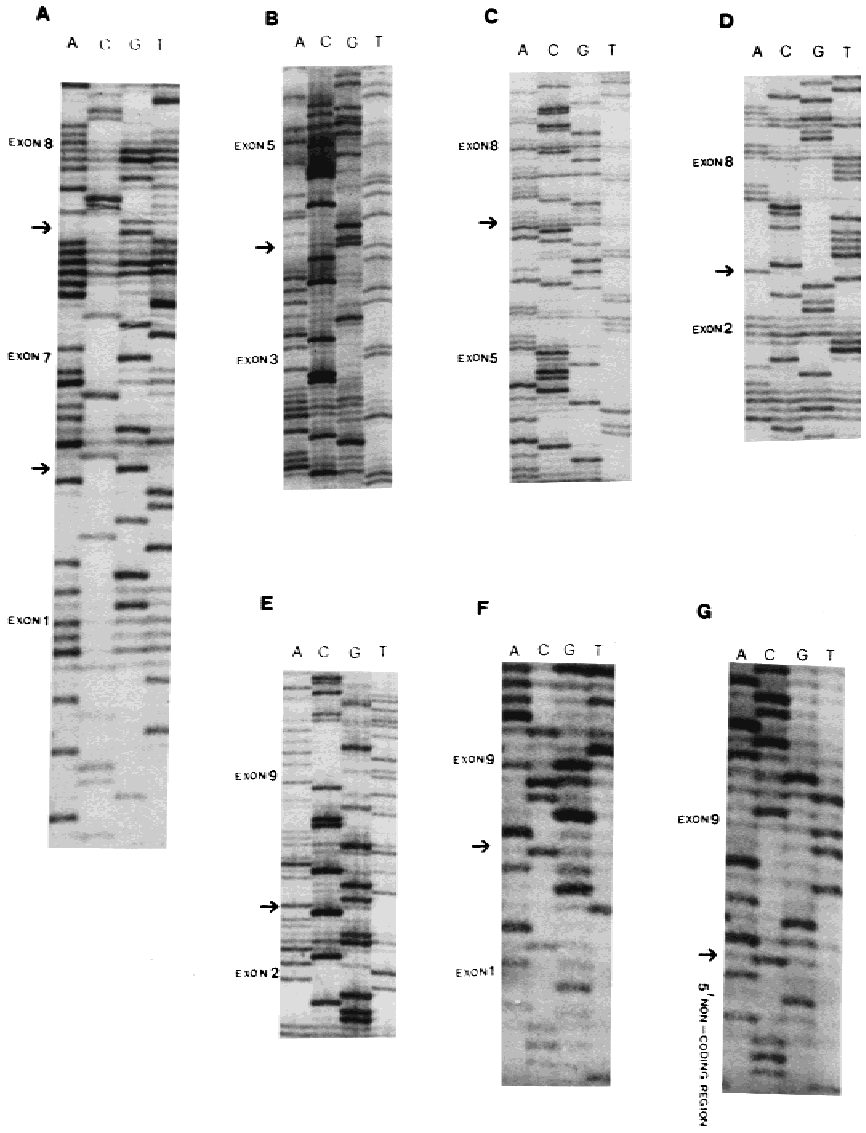

Fig. 2. The results of sequencing of the isolated fragments from 7 abnormal transcripts of the PTEN/MMAC1 gene. (A) Deletion from nt 1064 to 1814 and 1835 to 1877 of cDNA. (B) Deletion from nt 1244 to 1526 of cDNA. (C) Deletion from nt 1296 to 2013 of cDNA. (D) Deletion from nt 1170 to 2041 of cDNA. (E) Deletion from nt 1180 to 2142 of cDNA. (F) Deletion from nt 1040 to 2091 of cDNA. (G) Deletion from nt 1035 to 2165. Arrow indicates deletions.

M1u and M1d. The PCR products were diluted 20-fold, and 1 μL of the diluted product was used for a second round of PCR for 35 cycles using two nested primers, M2u and M2d. PCR amplifications were carried out as described previously [15]. All RT-PCR products were then subjected to direct sequencing analysis. The primers, M2u, M2d, and M3u, as described in Table I, were used for sequencing analysis. All the reactions were repeated at least three times with controls.

LOH Analysis

To detect allelic losses, the polymorphic short tandem repeat markers used were D10S215, D10S541, D10S579, and AFM086 [10]. The PCR amplification was performed in a volume of 25 μL containing 0.5 μg of genomic DNA, 2.5 pmol each of the primers, 5 μCi ^{35}S-dATP, 0.2 mM each of dNTPs, 1.25U AmpliTaq Gold™ DNA polymerase, and 1× PCR buffer. The PCR reactions were carried out for 35 cycles under the following conditions: 1.5 min at 94°C for denaturation, 1.5 min at 52–56°C for annealing, 2 min at 72°C for extension, and a final extension at 72°C for 5 min. PCR products were separated by electrophoresis at 70 W for 2 hr in 6% denaturing polyacrylamide gels containing 7 M urea. Gels were dried and autoradiographed for 6–18 hr.

PCR-SSCP Analysis

Oligonucleotide primers for amplification of exons 1–9 of PTEN/MMAC1 are shown in Table I. PCR was performed for 35 cycles, and then 5 μL of each of the amplicons were denatured in 2 μL denaturing solution (95% formamide, 10 mM EDTA, 0.05% bromophenol blue, 0.05% xylenecyanol) at 95°C for 5 min and thereafter directly placed on ice. A 5-μL aliquot of each sample was loaded, and electrophoresis was carried out in a GenePhor Electrophoresis Unit using the GeneGel

Excel 12.5/24 Kit. Staining was performed in a Gene Stain™ Automated Gel Stainer using PlusOne™ Silver Staining Kit (Pharmacia Biotech).

Sequence Analysis

Abnormal bands detected by SSCP analysis were analyzed for mutation by direct sequencing. The amplicons were purified using a gel extraction kit (Geneclean III Kit, Bio101, Inc., Vista, CA) and then sequenced using SequiTherm EXCEL™ II DNA Sequencing Kit (Epientre® Technologies, Madison, WI). The sequencing primers were the same as those used for the PCR-SSCP.

RESULTS

RT-PCR and cDNA Sequencing Analysis

The first PCR products were 1476 bp in length, and the length of nested PCR products was 1344 bp. Of the 62 AML specimens analyzed, 15 (24%) revealed the occurrence of aberrant PTEN/MMAC1 transcripts; a normal-sized band was present in all specimens (Fig. 1A). Sequencing analysis of the aberrant fragments of the PTEN/MMAC1 gene revealed the existence of eight types of RT-PCR products with losses of various exons of the published PTEN/MMAC1 sequence from exons 1–9 (Fig. 2). All of the deletions in aberrant transcripts involved exon 5 that contains the PTPase core motif. Two cases showed a complex deletion from exons 1 to 8 but retained 21 bp of exon 7 (Fig. 2). A product with exons 4–6 missing and an insertion of 51 bp was expressed in one specimen. Of the 15 cases that showed aberrant transcripts, 12 cases displayed one abnormal transcript and 3 had two abnormal transcripts (Fig. 1A). Four of the 5 cell lines (80%) displayed the aberrant transcripts; all 5 displayed the normal transcript (Fig. 1B). In addition, four of the 30 normal volunteers (13%) exhibited both a normal and an aberrant fragments (Fig. 1A). One control had a deletion of nucleotides (nt) 1064 through 1814, the others had deletion of nt 1180–2142, nt 1244–1526, nt 1296–2013, and nt 1835–1877. All of these patterns were found in our AML patients.

Sequencing of the normal-sized RT-PCR products amplified from the AML specimens, cell lines, and normal controls showed wild-type PTEN/MMAC1 transcripts except in the U937 line (4-base insertion) (data not shown).

Allelic Losses of PTEN/MMAC1 Gene Characterized by Loss of Heterozygosity Analysis

A total of 20 AML patients were analyzed for LOH using four microsatellite markers near or within the PTEN/MMAC1 gene locus. Microsatellite markers D10S215, D10S541, D10S579, and AFM086 displayed 10/20, 9/20, 12/20, and 8/20 heterozygosities, respectively. But none of 20 AML cases displayed LOH in these markers, suggesting that the regions defined by these markers had both alleles intact in these cases. We also screened for homozygous deletion at PTEN/MMAC1 gene by PCR amplification of all exons, but no homozygous deletion was observed in any of the AML cases or 5 cell lines.

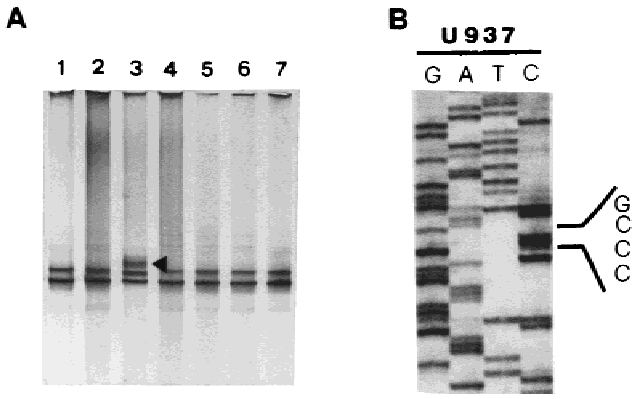

Fig. 3. (A) SSCP analysis of PTEN/MMAC1 exon 5. Altered mobility band (arrowhead) present in PCR products amplified from the leukemia cell line U937 DNA is seen in lane 3. (B) The result of sequencing of the altered band shows a 4-bp (CCCG) insertion at nucleotide 1423.

Mutational Analysis of PTEN/MMAC1 by PCR-SSCP and Direct Sequencing

We screened all 9 coding exons for coding sequence changes that would result in a defect of the protein (small deletion, insertion, or point mutation) by PCR-SSCP and direct sequencing. Genomic DNA samples from 62 AML patients and 5 cell lines were examined. PCR-SSCP analysis of exon 5 showed abnormal bands in the cell line U937 (Fig. 3A). Direct sequencing of the aberrant band showed an insertion of 4 bases (CCCG) at nt 1423 of exon 5 (Fig. 3B). The insertion resulted in the frameshift mutation of codon 130 with premature termination of the coding sequence. No mutations were observed in any of the AML or other tumor cell lines.

DISCUSSION

PTEN/MMAC1 is a putative tumor suppressor gene recently identified at chromosome 10q23.3, which contains 9 exons and encodes a 403 amino acid protein [9,10]. The PTEN/MMAC1 protein is a dual-specificity phosphatase containing a protein tyrosine phosphatases catalytic domain and a region homology with the cytoskeletal proteins tensin and auxillin [9,11,16]. Protein tyrosine phosphatases have been thought to play a role in tumor suppression due to antagonize the activity of the protein tyrosine kinase. The somatic deletions and mutations of PTEN/MMAC1 gene were identified in several cancer cell lines and primary cancers including glioma,

breast, endometrium, prostate, thyroid, and malignant melanoma [9,10,17–25]. The loss of function for PTEN/MMAC1 appears to occur during the progression of multiple human cancers. PTEN/MMAC1 has also been identified as the gene responsible for Cowden disease, a rare familial syndrome associated with an elevated risk for tumors of the breast, thyroid, and skin, and Bannayan-Zonana syndrome, a related harmartomatous polyposis syndrome [14,26,27].

In the current study, we found aberrant PTEN/MMAC1 RT-PCR products in 24% of AML patients, 80% of hemopoietic cell lines, and 13% of normal controls. All of the aberrant transcripts with intragenic deletion of exon 5 contained the PTPase core motif. Aberrant transcripts from our normal controls all had the same deletion locations as the AML patients, although aberrant transcripts occurred more frequently in the AML specimens and cell lines. The abnormal transcripts may result from aberrant RNA splicing, as evidenced by the occurrence of aberrant transcripts in the AML specimens, cell lines, and normal controls, and the simultaneous presence of normal full length transcripts in all tumor samples and cell lines examined. This result is consistent with Gayther's study, Chang's study, and our previous study of TSG101 and FHIT [15,28–30], in which aberrant transcriptions with different deletions were frequently identified. However, PCR amplification of aberrant transcripts caused by pre-mRNA splicing that was less stringent in cancer cells than in normal tissues should be considered [31].

In addition, most of the aberrant transcripts of PTEN/MMAC1 were caused by intragenic deletions. This result differs from our previous studies of FHIT and TSG101 [15,32], in which deletions were located at the exon–intron junction region. The significance of these differences remains to be elucidated. Whether the aberrant transcripts in these candidate tumor suppressor genes are important in leukemogenesis or are merely the consequence of splicing infidelity in leukemic cells needs further investigation.

LOH analysis in our 20 AML cases (all lacked chromosome 10 or 10q deletions) with hair follicle constitutional DNA showed that regions defined by markers D10S215, D10S541, D10S579, and AFM086 were intact in both alleles. Mutational analysis of the entire 9 coding exons showed that only one cell line (U937) had a frameshift mutation resulting in premature termination of the PTEN/MMAC1 protein. None of the AML cases had mutations. This result is different from Sakai's study of lymphoid neoplasms, in which the abnormalities of the PTEN/MMAC1 gene may contribute to pathogenesis in a small percentage of malignant lymphoma [33]. Our results are also different from McGlynn study and Padua study of RAS, FMS, and p53 [34,35], in which the loss of FMS gene, mutation of RAS, FMS, and p53 were common in AML and myelodysplasias. Our data indicate that the PTEN/MMAC1 gene may not play a major role in AML. However, due to the small number cases of our study and AML is a wide diversity disease which may differ in etiology, pathology, and behavior, the potential role of PTEN/MMAC1 in AML or in some subtypes of AML need further investigation.

REFERENCES

1. Bishop JM. The molecular genetics of cancer. Science 1987;235:305.
2. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AM, Bos JL. Genetic alterations during colorectal-tumor development. N Engl J Med 1988;319:525.
3. Knudson AG. Genetics of human cancer. Annu Rev Genet 1989;20:231.
4. Cline MJ. The molecular basis of leukemia. N Engl J Med 1994;330:328.
5. Mitelman F. Catalog of chromosomal aberrations in cancer. 5th edition. New York: Wiley-Liss; 1994.
6. Rabbits TH. Chromosomal translocations in human cancer. Nature 1994;372:143.
7. Marshall CJ. Tumor suppressor genes. Cell 1991;64:313.
8. Yokota J, Sugimura T. Multiple steps in carcinogenesis involving alterations of multiple tumor suppressor genes. FASEB J 1993;7:920.
9. Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, Puc J, Miliaresis C, Rodgers L, McCombie R, Bigner SH, Giovanella BC, Ittmann M, Tycko B, Hibshoosh H, Wigler MH, Parsons R. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science 1997;275:1943.
10. Steck PA, Pershouse MA, Jasser SA, Yung WK, Lin H, Ligon AH, Langford, LA, Baumgard ML, Hattier T, Davis T, Frye C, Hu R, Swedlund B, Teng DHF, Tavtigian SV. Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nat Genet 1997;15:356.
11. Myers MP, Stolarov JP, Eng C, Li J, Wang SI, Wigler MH, Parsons R, Tonks NK. PTEN, the tumour suppressor from human chromosome 10q23, is a dual-specificity phosphatase. Proc Natl Acad Sci USA 1997;94:9052.
12. Teng DH-F, Hu R, Lin H, Davis T, Iliev D, Frye C, Swedlund B, Hansen KL, Vinson VL, Gumpper KL, Ellis L, El-Naggar A, Frazier M, Jasser S, Langford LA, Lee J, Mills GB, Pershouse MA, Pollack RE, Tornos C, Troncoso P, Yung WKA, Fujii G, Berson A, Bookstein R, Bolen JB, Tavtigian SV, Steck PA. MMAC1/PTEN mutation in primary tumor specimens and tumor cell lines. Cancer Res 1997;57:5221.
13. Liu TC, Lin SF, Chen TP, Liu HW, Chang JG. Mutation analysis of the ras gene in myelocytic leukemia by polymerase chain reaction and oligonucleotide probes. J Formos Med Assoc 1991;90:825.
14. Lin SF, Liu TC, Chen TP, Chiou SS, Liu HW, Chang JG. Diagnosis of thalassaemia by non-isotope detection of α/β and δ/β mRNA ratios. Br J Haematol 1994;87:133.
15. Lin PM, Liu TC, Chang JG, Chen TP, Lin SF. Aberrant TSG 101 transcripts in acute myeloid leukaemia. Br J Haematol 1998;102:753.
16. Furnari FB, Lin H, Huang HS, Cavence WK. Growth suppression of glioma cells by PTEN requires a functional phosphatase catalytic domain. Proc Natl Acad Sci USA 1997;94:12479.
17. Ahmed Rasheed BK, Stenzel TT, McLendon RE, Parsons R, Friedman AH, Friedman HS, Bigner DD, Bigner SH. PTEN gene mutations in high-grade but not in low-grade gliomas. Cancer Res 1997;57:4187.
18. Cairns P, Okami K, Halachmi SH, Halachmi N, Esteller M, Herman

JG, Jen J, Issacs WB, Bova GS, Sidransky D. Frequent inactivation of PTEN/MMAC1 in primary prostate cancer. Cancer Res 1997;57:4997.
19. Dahia PLM, Marsh DJ, Zheng Z, Zedenius J, Komminoth P, Frisk T, Wallin G, Parsons R, Longy M, Larsson C, Eng C. Somatic deletions and mutations in the Cowden disease gene, PTEN, in sporadic thyroid tumors. Cancer Res 1997;57:4710.
20. Gulderberg P, Straten PT, Birck A, Ahrenkiel V, Kirkin AF, Zeuthen J. Disruption of the MMAC1/PTEN gene by deletion or mutation is a frequent event in malignant melanoma. Cancer Res 1997;57:3660.
21. Liu W, James CD, Frederick L, Alderete BE, Jenkins RB. PTEN/MMAC1 mutations and EGFR amplification in glioblastoma. Cancer Res 1997;57:5254.
22. Rhei E, Kang L, Bogomolniy F, Federici MG, Borgen PI, Boyd J. Mutation analysis of the putative tumor suppressor gene PTEN/MMAC1 in primary breast carcinoma. Cancer Res 1997;57:3657.
23. Risinger JI, Hayes AK, Berchuck A, Barrett JC. PTEN/MMAC1 mutations in endometrial cancers. Cancer Res 1997;57:4736.
24. Tashiro H, Blazes MS, Wu R, Cho KR, Bose I, Wang SI, Li J, Parsons R, Ellenson LH. Mutation in PTEN are frequent in endometrial carcinoma but rare in other common gynecological malignancies. Cancer Res 1997;57:3935.
25. Wang SI, Puc J, Li J, Bruce JN, Cairns P, Sidransky D, Parsons R. Somatic mutations of PTEN in glioblastoma multiforme. Cancer Res 1997;57:4183.
26. Liaw D, Marsh DJ, Li J, Dahia PL, Wang SI, Zheng Z, Bose S, Call KM, Tsou HC, Peacocke M, Eng C, Parsons R. Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. Nat Genet 1997;16:64.
27. Marsh DJ, Dahia PLM, Zheng Z, Liaw D, Parsons R, Gorlin RJ, Eng C. Germline mutations in PTEN are present in Bannayan-Zonana syndrome. Nat Genet 1997;16:333.
28. Gayther SA, Barski P, Batley SJ, Li L, de Foy KAF, Cohen SN, Ponder BAJ, Caldas C. Aberrant splicing of the TSG101 and FHIT genes occurs frequently in multiple malignancies and in normal tissues and mimics alterations previously described in tumours. Oncogene 1997;15:2119.
29. Chang JG, Chen YJ, Perng LI, Wang NM, Kao MC, Yang TY, Chang CP, Tsai CH. Mutation analysis of the PTEN/MMAC1 gene in the cancer of digestive tract. Eur J Cancer 1999;35:647.
30. Wang NM, Chang JG. Are aberrant transcripts of FHIT, TSG101, and PTEN/MMAC1 oncogenesis related? Int J Mol Med 1999;3:491.
31. Haber D, Harlow E. Tumor-suppressor genes: Evolving definitions in the genomic age. Nat Genet 1997;16:320.
32. Lin PM, Liu TC, Chang JG, Chen TP, Lin SF. Aberrant FHIT transcripts in acute myeloid leukaemia. Br J Haematol 1997;99:612.
33. Sakai A, Thieblemont C, Wellmann A, Jaffe ES, Raffeld M. PTEN gene alterations in lymphoid neoplasms. Blood 1998;92:3410.
34. McGlynn H, Kapelko K, Baker A, Burnette A, Padua RA. Allelic loss of the FMS gene in acute myeloid leukemia. Leuk Res 1997;21:919.
35. Padua RA, Guinn BA, Al-Sabah AI, Smith M, Taylor C, Pettersson T, Ridge S, Carter G, White D, Oscier D, Chevret S, West R. RAS, FMS and p53 mutations and poor clinical outcome in myelodysplasias: A 10-year follow-up. Leukemia 1998;12:887.